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Triaculetin, a New Diterpene from a Saudi Arabian Plant, *Euphorbia triaculeata*

Saboor Ahmad, Mohammad Jahangir and Mohsin Abdul Karim

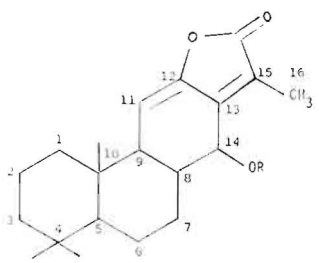
Analytical Chemistry Section,
Regional Agriculture and Water Research Center,
Ministry of Agriculture and Water,
P.O. Box 17285
Riyadh, Saudi Arabia 11484

ABSTRACT. As a result of continuing search among Saudi Arabian plants for new naturally occurring potential antitumor agents, an ethanolic extract of *Euphorbia triaculeata* Forssk. has met criteria for confirmed activity in PL-388 leukemia system. The active extract has led to the isolation and characterization of a new diterpene, Triaculetin. This paper describes the isolation and structural elucidation of the new diterpene.

Family EUPHORBIACEAE has been the subject of considerable interest for its antileukemic and co-carcinogenic compounds. It is rich in phorbol esters and ingenol esters or their derivatives to which the antileukemic and co-carcinogenic properties have been attributed (Kupchan *et al.* 1976, Schwendt and Hecker 1974). As a result of continuing search among Saudi Arabian plants for new naturally occurring potential antitumor agents, an ethanolic extract of roots of *Euphorbia triaculeata* Forssk. was found to show significant inhibitory activity *in vivo* against the P-388 lymphocytic leukemia in mice. We report now the structural elucidation of the new diterpenic constituent, triaculetin, isolated from the active extract.

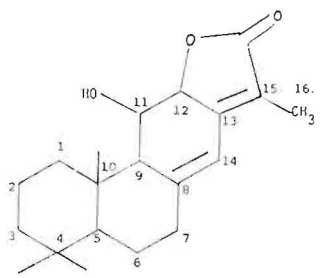
Results and Discussion

Roots of *E. triaculeata* collected from Jizan, in southern Saudi Arabia, were powdered and extracted by standard procedures set by the National Cancer Institute, U.S.A. The crude ethanolic extract which showed positive activity against P-388 type of leukemia was concentrated and chromatographed on a neutral alumina column



Triaculetin 1R = H

Acetate of Triaculetin 1R = Ac



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Dehydration Product of

Tetrahydrojolkinolide

which was eluted with benzene followed by gradual increase in polarity. Elution with chloroform gave a mixture of colourless material which was subjected to preparative TLC on alumina and eluted with 20% ethyl acetate in benzene to give colourless needles of triaculetin from pet. ether m.p. 198-200°C. It had the following spectral data:

Mass spectra showed M^+ at m/e 316 ($C_{20}H_{28}O_3$). Elemental analyses confirmed its mol. form. as $C_{20}H_{28}O_3$ (required: C = 75.91, H = 8.92; Found: C = 75.78, H = 8.80), IR (KBr) cm^{-1} 1725 (lactone C= O), 1605, 1660 (C = C), 3470 (OH). NMR δ ($CDCl_3$) 0.82, 0.88, 0.99 (9H, S, Methyls at 4,4′, 10), 1.81 (3H,d, J = 1.5 Hz, 16-CH_3), 3.95 (1H, d of q J = 1.5 Hz and 9.3 Hz, 14-H), 6.25 (1H, d, J = 6 Hz, 11-H), 3.60 (1H, m, 8-H), 2.32 (2H, m, 9-H and 14-OH), UV λ_{max} (MeOH) 275 nm (ϵ = 17000).

The chemical shifts of the three tertiary methyl groups and the conjugation pattern shown by UV were reminiscent of the diterpenoids having abietane skeleton particularly in the Jolkinolides and Caudicifolin (Ahmad *et al.* 1977, Uemura and Hirata 1972). The presence of the only secondary alcoholic group was confirmed by the formation of the monoacetate 1R = Ac, IR (Nujol) 1755 cm^{-1} . The OH was masked by the 9-H multiplet in the NMR spectrum. This was shown by decrease in the intensity of the multiplet by addition of CF_3 COOH. The presence of conjugated diene was shown by the UV spectrum. The above spectral data supports structure 1R – H or 2. The 16-CH_3 doublet in the NMR spectrum is typical of a methyl split with the proton in 12 position of structure 2 or split with 14-H as in structure 1R = H (Uemura and Hirata 1972). That the methyl was split by 14-H was shown by irradiation studies which confirmed the presence of OH at 14 position and the double bond at 11-12 position. These results were confirmed by double resonance studies on a 100 MHZ NMR. In addition, the compound having structure 2 had already been described in literature (Uemura and Hirata 1972), the physical data of which particularly the NMR of 2 is distinctly different from 1R = H. We, therefore, propose 1R = H as the only alternative structure of the compound triaculetin. The work on the stereochemistry of the compound is in progress.

Experimental

Melting points are uncorrected. Proton NMR obtained on a varian (60 MHZ) or Jeol FX 100 (100 MHZ) using $CDCl_3$ as solvent. The chemical shift is presented in (ppm) using the internal standard (TMS). Mass spectra were recorded on a Ripermag R-1010 by direct inlet at 70 ev. IR were recorded in Nujol mull on the Pye unicam instrument SP 2000. UV was recorded in ethyl alcohol on the Beckman ACTA, M VI.

Dried rootes of *Euphorbia triaculeata* (1 kg) were defattened with pet. ether 60-80°C followed by extraction with hot 95% ethyl alcohol in a soxhlet apparatus. Ethanol was removed under reduced pressure and residue concentrated to a thick paste which was dissolved in chloroform and chromatographed on a neutral alumina column. Elution of column with pet. ether and benzene mostly gave fatty material. Further elution with benzene: chloroform (1:1) followed by pure chloroform gave a colourless semi-crystalline mixture. It was subjected to preparative thin layer chromatography using benzene:ethylacetate (8:2) as eluent. The flourescent band, which had an R_f value of 0.45, was extracted with chloroform. Removal of chloroform and crystallization of the residue with pet. ether gave colourless needles (m.p. 198-200°C, 15 mg) of triaculetin.

MS: M^+ at 316 ($C_{20}H_{28}O_3$)
Required: C = 75.91, H = 8.92%
Found: C = 75.78, H = 8.80%
IR ν (KBR): 1725, 1605, 1660, 3470 cm^{-1}
NMR δ ($CDCl_3$): 0.82, 0.88, 0.99 (9H,S), 1.81 (3H,d, J = 1.5 Hz), 3.95 (1H, d of q J = 1.5 Hz and 9.3 Hz),6.25 (1H,d, J = 6 Hz), 3.60 (1H, m), 2.32 (2H,m).
UV λ_{max} (MeOH): 275 nm (ϵ = 1700)
Monoacetate formed by usual treatment with pyridine and acetic anhydride m. p. 162-164°. IR (Nujol) 1755 cm^{-1}.

Acknowledgement

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دايتربين جديد من نبات يوفوربيا ترايكيوليتا في المملكة العربية السعودية

صبور أحمد، محمد جهانقير، عبد المحسن العبد الكريم
المركز الإقليمي لأبحاث الزراعة والمياه بالرياض
الرياض ص. ب. ١٧٢٨٥ ـ المملكة العربية السعودية

بناء على نتائج الأبحاث الجارية على النباتات الطبيعية في المملكة العربية السعودية ولنبات يوفوربيا ترايكيوليتافورسك، وجد أنها تحتوي على مواد مقاومة للأورام الخبيثة. وقد تم التحقق من تلك الحالة من خلال الدراسة على مرض (بال ٣٨٨ ليوكوميا):

ومن خلال البحث على المستخلص الكحولي لها (الإيثانولي) وجد أنها تحتوي على مركب جديد اسمه ترايكيولتين يتبع مركبات الدايتربين.

يوضح هذا البحث كيفية فصل ذلك المركب ويشرح تركيبه الكيميائي.